

Direct rapid prototyping of PDMS from a photomask film for micropatterning of biomolecules and cells

Hyundoo Hwang,^{‡a} Gyumin Kang,^{‡a} Ju Hun Yeon,^a Yoonkey Nam^{*a} and Je-Kyun Park^{*ab}

^aDepartment of Bio and Brain Engineering, KAIST, 335 Gwahangno, Yuseong-gu, Daejeon 305-701, Korea

^bDepartment of Biological Sciences, KAIST, 335 Gwahangno, Yuseong-gu, Daejeon 305-701, Korea

*Corresponding authors. Department of Bio and Brain Engineering, KAIST, 335 Gwahangno, Yuseong-gu, Daejeon 305-701, Korea. Fax: +82 42 350 4310; Tel: +82 42 350 4315; jekyun@kaist.ac.kr (J.-K. Park). Tel.: +82 42 350 4322; ynam@kaist.ac.kr (Y. Nam),

[‡]Contributed equally.

Cell culture method

Human hepatocellular carcinoma cells and fibroblasts. The human hepatocellular carcinoma cell line (HepG2/C3A, ATCC CRL-10741) and human skin fibroblast (CCD-986sk, ATCC CRL-1947) were selected for cell patterning with PLL-FITC on glass. The cell layers were briefly rinsed with PBS with a pH of 7.4 (Gibco, Grand Island, NY). Then, the trypsin-EDTA solution (0.25% trypsin and 1 mM EDTA·4Na; Gibco) was used to detach the cells at 78%-80% confluent cell layer and the Dulbecco's modified Eagle's medium (DMEM; Gibco) with 10% fetal bovine serum (FBS, Gibco) was supplemented to the dispersed cell layer. The cell cultures were maintained at 37 °C under 5% CO₂ in a humidified water-jacketed incubator. The appropriate aliquots of the cell suspension was refreshed and diluted to suitable concentrations of 1×10⁶ cells/ml. Before cell spreading on culture dish, the PLL-FITC patterned glass was sterilized with 70% ethanol and cell culture media was changed after 1 h for cell attachment.

Hippocampal neurons. Primary hippocampus dissected from E-18 Sprague-Dawley rat embryos was incubated with trypsin 2.5% for 15 min in a 37 °C water bath. After rinsing residual trypsin with Hank's Buffered Salt Solution (HBSS), hippocampus was triturated using fire-polished Pasteur pipette in Neurobasal medium (Invitrogen, CA) supplemented with B27 (Invitrogen), 2 mM L-glutamine (Invitrogen), 12.5 μM glutamate (Sigma-Aldrich) and penicillin-streptomycin (Invitrogen). Dissociated neurons were centrifuged at 1000 rpm for 3 min. Supernatant was removed and pellet was triturated again by Pasteur pipette with 1 ml of fresh medium. Then neurons were seeded on the sterilized substrate at the density of 100 cells/mm². Plated neurons were kept in a humidified incubator with 5% CO₂ and 37 °C for 3 days in vitro.